FORMULATION, EVALUATION AND COMPARISION OF LIPOSOMES OF *BAUHINIA VARIEGATE Benth* AND *MACROTYLOMA UNIFLORUM LAM Verdc* FOR NEPHROPROTECTIVE ACTIVITY

Synopsis

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INTRODUCTION

Liposomes are round bubbles consisting of an aqueous core encapsulated by natural or synthetic phospholipids. This structure turns liposomes into ideal drug carriers, since hydrophilic drugs tend to be entrapped in the core; while hydrophobic ones will be entrapped within the lipid bilayers. The encapsulation is partially dependent on the partition coefficient of a certain drug[1]. Prepared by different methods, liposomes vary with size, lamellarity and surface charge. Generally, liposomes can be classified as unilamellar or multilamellar. Unilamellar liposomes are further divided into small size (SUV, 50–100 nm) or large size (LUV, 100–250 nm). SUV and LUV are both composed of a single lipid bilayer and a large aqueous core, thus suitable for loading hydrophilic drugs; while multilamellar liposomes (MLV), usually with a diameter of 1–5 μm, are composed of several lipid bilayers and a limited aqueous space, thus suitable for loading hydrophobic drugs.

Nephrotoxicity is one of the most common kidney problem and occurs when body is exposed to a medicine or chemicals. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome because increasing number of potent therapeutic drugs like aminoglycoside antibiotics, chemotherapeutic agents and NSAIDS. Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances.

Demand for herbal plants is increasing in both developing and developed countries. Kidney failure is one of the most common diseases from a lot of accepted diseases in India. Many plants accept been acclimated for the analysis of Kidney failure in acceptable arrangement of anesthetic throughout the world. Indeed along with dietary measures, plant preparation formed the base of the treatment of the ache until the accession of allopathic medicine[2]. Ethno-medicinal plants can be acclimated to advice apprehend the charge for dialysis by treating the causes and aftereffect of renal failure, as well as reducing the many adverse effect of dialysis. Nephrotoxicity can be authentic as renal disease or dysfunction that arises as a absolute or aberrant aftereffect of exposure to medicines, and environmental or industrial chemicals. Several factors accept been articular which accomplish the kidney accessible to toxic injury due to indigenous medicines. This includes urine pH, High blood flow rate, high endothelial surface area, high metabolic activity, active uptake by tubular cell and medullary interstitial concentration. The toxins may

abuse the tubules directly, at the website of adulteration carriage or concentration, or by inducing renal ischemia, hemoglobinuria or myoglobinuria[3]. Continued acknowledgment and acknowledgment to top doses can access the severity of renal failure. The Nephrotoxic effect of cyclosporine, aminoglycoside antibiotics, cisplatin, amphotericin-B, beta-lactam antibiotics and Indomethacin are reviewed. These drugs were produce produced nephrotoxicity because they are most frequently causes of renal injury in children. In addition, their nephrotoxicity is acquired by altered mechanisms. Several generalizations can be made, however. First, agents which could cause tubular accident tend to be accessory in their baneful effects. This review attempts to portray the discory and development of anesthetic from galenical to genomical, with a focus on the abeyant and role of medicinal plants. Ayurveda is a acceptable Indian system of medicine getting accomplished for thousands of years. Ethnomedicinal studies are generally cogent in absolute locally important bulb breed abnormally for the analysis of awkward drug[4].

DIFFERENT TYPES OF NEPHROTOXICITY

Aminoglycoside nephrotoxicity: Aminoglycosides specially influence the proximal tubular cells. These operators are uninhibitedly separated by the glomeruli and immediately taken up by the proximal tubular epithelial cells, where they are consolidated into lysosomes after first connecting with phospholipids on the brush fringe films. They apply their primary harmful impact inside the tubular cell by adjusting phospholipid digestion system. Notwithstanding their immediate impact on cells, aminoglycosides cause renal vasoconstriction. The basic figures the improvement of acute kidney Injury (AKI) auxiliary to aminoglycoside nephrotoxicity are dosing and term of treatment. Aminoglycoside take-up by the tubules is a saturable marvel, so take-up is restricted after a solitary measurement. Consequently, a solitary every day huge measurement is desirable over 3 dosages for each day. One measurement for each day probably causes less amassing in the tubular cells once the immersion point is reached[5].

Amphotericin B nephrotoxicity: Amphotericin B ties to sterols in cell films, in this way making pores that bargain layer uprightness and increment layer penetrability. It ties to ergosterol in contagious cell dividers as well as to cholesterol in human cell films; this is the thing that records for its nephrotoxicity. Trademark electrolyte variations from the norm incorporate squandering of potassium and magnesium auxiliary to expanded penetrability of the phone layers. The back-break of hydrogen particles in the gathering conduit prompts to distal renal tubular acidosis (dRTA). Lipid-based arrangements Srivastava et al Der Pharmacia Sinica,

2017, 8(1):11-24 Pelagia Research Library 13 of amphotericin B diminish however don't wipe out the nephrotoxicity contrasted and customary amphotericin B. This might be because of a direct nephrotoxic impact of the traditional preparations. Contrast-induced nephropathy In spite of the fact that the pathogenesis of contrast-induced nephropathy (CIN) remains not entirely comprehended, it is doubtlessly the consequence of renal vasoconstriction and direct renal tubular epithelial cell poisonous quality. Current hypotheses in regards to CIN danger incorporate a mix of direct cytotoxicity with postischemic reperfusion harm bringing about oxygen free radical creation prompting to endothelial damage[6].

Calcineurin inhibitor nephrotoxicity: Cyclosporine and tacrolimus cause acute kidney injury (AKI) by initiating afferent and efferent arteriolar vasoconstriction. Steady harm can prompt to interstitial fibrosis. Tacrolimus has been appeared to bring about thrombotic microangiopathy therefore of endothelial injury.

Cisplatin nephrotoxicity: Cisplatin more often than not influences the proximal tubules principally with some optional impact on the glomeruli and distal tubules. Cisplatin is discharged basically in the pee, bringing about extracted medication levels, which energize take-up into the phones by uninvolved dispersion or dynamic take-up. Cisplatin is steady in the circulatory system yet gets to be hydrolyzed in the chloride-poor cell condition. It is the hydrolyzed metabolite that ties DNA, RNA, proteins, and phospholipids, bringing on cytotoxicity.

Ifosfamide nephrotoxicity: Ifosfamide is a known simple of cyclophosphamide. Despite the fact that cyclophosphamide is not nephrotoxic, ifosfamide, by ethicalness of its metabolite chloroacetaldehyde, is harmful to the tubular cells, with special association of the proximal tubule prompting to Fanconi syndrome[7].

Foscarnet nephrotoxicity: Foscarnet, which is utilized to treat safe cytomegalovirus (CMV) diseases, causes intense interstitial nephritis and intratubular precious stone arrangement. Notwithstanding precious stone arrangement, which can be comprised of calcium salts or sodium salts, chelation of calcium by foscarnet prompts to hypocalcemia. Crystal-forming drug nephrotoxicity Sulfa drugs, acyclovir, methotrexate, ethylene glycol, and protease inhibitors like indinavir cause acute kidney injury (AKI) by tubular impediment because of precious stone development in the tubular cells. Acyclovir may prompt to the development of intratubular precious stones, which show up as birefringent needle-molded gems and can evoke an intense interstitial nephritis[8].

Rhabdomyolysis: Rhabdomyolysis alludes to the breakdown of skeletal muscle strands, which prompts to the arrival of conceivably nephrotoxic intracellular substance into the course[17].

Acute kidney injury (AKI) creates in this setting by means of the accompanying 3 instruments: a) Renal vasoconstriction. b) Heme-interceded proximal tubular cell toxicity. c) Intratubular cast arrangement.

Clinical features

Pain is the most common presenting symptom of ureteric calculi and is caused by the stone obstructing the urinary tract. A sudden onset of acute loin pain, often at night when the urine is maximally concentrated. A stone can become impacted along the urinary tract at one of the five anatomical locations. Classical renal colic is characterized by an acute onset of pain in the loin that radiates to the groin and scrotum or labia majora.

Infection may be a causal factor in stone formation or may be secondary to obstruction caused by the calculus. Typically, infection with urease-producing organisms causes alkalinization of the urine, leading to formation of magnesium ammonium phosphate stones which may become large staghorn

Haematuria: blood in the urine, due to minor damage to inside wall of kidney, ureter and/or urethra. Haematuria is present in 85–90 % of patients with stone and is usually microscopic (although frank blood may be observed). Haematuria may be absent (even on dipstick analysis) in up to 15% of patients.

Pyuria: pus in the urine.

Dysuria: burning on urination when passing stones (rare). More typical of infection.

Oliguria: reduced urinary volume caused by obstruction of the bladder or urethra by stone or extremely rarely, simultaneous obstruction of both ureters by a stone.

Nausea/vomiting: Embryological link with intestine stimulates the vomiting center.

Fever and chills.

Hydronephrosis

Postrenal azotemia: when kidney stone blocks ureter[9].

MAIN METABOLIC ABNORMALITIES IN PATIENTS WITH RENAL FAILURE

1. Anorexia–reduced oral nutrient intake.

2. Gastrointestinal consequences of uraemia.

3. Restrictive diets.

4. Uremic toxicity–inadequate dialysis prescription.

5. Metabolic acidosis.

6. Endocrine factors (PTH, insulin resistance etc.).

7. Peripheral insulin resistance.

8. Impairment of lipolysis.

9. Low grade inflammatory state activation of protein catabolism.

10. Augmented catabolic response to intercurrent disease.

11. Metabolic acidosis.

12. Hyperparathyroidisms, uremic bone disease.

13. Impairment of vitamin D3 activation.

Diagnosis of Nephrolithiasis

A. Urinalysis

Urine analysis by microscopy in a patient with renal colic due to a stone usually shows the presence of blood. However, up to 15% of patients test negative. Sometimes stone-forming crystals can be seen in the urine.

Table 1: List of analyses carried out as part of a Stone Screen procedure

Sample	Analyses performed
Stone	Quantitative infra-red analysis for mineral constituents
Blood	Urea, creatinine, sodium, potassium, bicarbonate, albumin, calcium, magnesium, phosphate, urate, alkaline phosphatase. PTH and 25(OH)-vitamin D, oxalate (in cases of severe hyperoxaluria)
Spot urine	Creatinine, urea, pH, calcium, phosphate, oxalate, urate, sodium, potassium, magnesium
24-h urines	Volume, pH, creatinine, calcium, magnesium, phosphate, oxalate, citrate, urate, urea, sodium, potassium, protein, qualitative cystine (and quantitative cystine), ornithine, arginine and lysine, if

	qualitative cystine is positive
Diet diary	Fluids, water, calories, calcium, magnesium, phosphate, oxalate, total protein, animal protein, meat + fish + poultry protein, fruit + vegetable + cereal protein, dairy protein, purine, fat, refined carbohydrates, fibre, sodium and potassium

(Source: Renal stone disease. Medicine 35:8, 418)

B. Imaging (Radiography)

- **A kidney-ureter-bladder (KUB) X-ray**
- **Ultrasound**
- **Intravenous urogram (IVU)**
- **Computed tomography (CT KUB)**

X-rays

The relatively dense calcium renders these stones radio-opaque and they can be detected by a traditional X-ray of the abdomen that includes the Kidneys, Ureters and Bladder (KUB). This may be followed by an IVP (Intravenous Pyelogram also called IntraVenous Urogram (IVU)) which requires about 50 ml of a special dye to be injected into the bloodstream that is excreted by the kidneys and by its density helps outline any stone on a repeated X-ray. About 10% of stones do not have enough calcium to be seen on standard x-rays (radiolucent stones).

Computed tomography

Computed tomography without contrast is considered the gold-standard diagnostic test for the detection of kidney stones. All stones are detectable by CT except very rare stones composed of certain drug residues in the urine. If positive for stones, a single standard x-ray of the abdomen (KUB) is recommended. This gives a clearer idea of the exact size and shape of the stone as well as its surgical orientation. Further, it makes it simple to follow the progress of the stone by doing another x-ray in the future. Drawback of CT scans include radiation exposure and cost.

Ultrasound

Ultrasound imaging is useful as it gives details about the presence of hydronephrosis (swelling of the kidney suggesting the stone is blocking the outflow of urine). It can also be used to detect stones during pregnancy when x-rays or CT are discouraged. Radiolucent stones may show up on ultrasound however they are also typically seen on CT scans. Some recommend that US be used as the primary diagnostic technique with CT being reserved for those with negative US result and continued suspicion of a kidney stone. This is due to its lesser cost and lack of radiation exposure.

Isotope renography

It involves intravenous administration of a radiopharmaceutical agent (labelled with technetium-99) and imaging with a gamma camera that allows assessment of uptake, transit and elimination by the kidney. Information about the relative function of the kidneys, the degree of obstruction and monitoring of changes in function of an obstructed kidney can be obtained, but depends on the radiopharmaceutical used. Function and obstruction can be assessed with dynamic renography using diethylenetriamine pentaacetic acid and mercaptoacetyltriglycine, whereas relative renal function is assessed using static renography with dimercaptosuccinic acid[10].

Others:

Other investigations typically carried out include:

- **Microscopic study of urine**, which may show proteins, red blood cells, bacteria, cellular casts and crystals.
- Culture of a urine sample to exclude urine infection (either as a differential cause of the patient's pain, or secondary to the presence of a stone)
- **Blood tests**: Full blood count for the presence of a raised white cell count (Neutrophilia) suggestive of infection, a check of renal function and to look for abnormally high blood calcium blood levels (hypercalcaemia)[11].
- Catching of passed stones at home (usually by urinating through a tea strainer or stone screen) for later examination and evaluation by a doctor.

- **Intravenous urography (IVU)** involves intravenous injection of contrast medium and plain radiography.
- **MRI**

Pathophysiology of nephrotoxicity

The kidney consists of numerous cell types organized into the nephron, which is the basic functional unit of the kidney. Any stimuli that induce loss of these cells can induce kidney damage and renal failure. The cause of renal failure can be intrinsic or extrinsic. Extrinsic causes include cardiovascular disease, obesity, diabetes, sepsis, and lung and liver failure. Intrinsic causes include glomerular nephritis, polycystic kidney disease, renal fibrosis, tubular cell death, and stones. The kidney plays a prominent role in mediating the toxicity of numerous drugs, environmental pollutants and natural substances. Drugs known to be nephrotoxic include several cancer therapeutics, drugs of abuse, antibiotics, and radiocontrast agents. Environmental pollutants known to target the kidney include cadmium, mercury, arsenic, lead, trichloroethylene, bromate, brominated-flame retardants, diglycolic acid, and ethylene glycol. Natural nephrotoxicants include aristolochic acids and mycotoxins such as ochratoxin, fumonisin B_1, and citrinin. There are several common characteristics between mechanisms of renal failure induced by nephrotoxicants and extrinsic causes. This common ground exists primarily due to similarities in the molecular mechanisms mediating renal cell death.

There are so many plants with nephroprotective activity like Bauhinia variegate, Benincasa hispida, Brassica nigra, Brassica rapa, Bryophyllum calycinum, Carum carvi, Cassia occidentalis, Casuarina equisetifolia, Citrullus colocynthis, Crocus sativus, Cuminum cyminum, Cymbopogon schoenanthus, Cynodon dactylon, Daucus carota, Foeniculum vulgare, Moringa oleifera, Oxalis corniculata, Macrotyloma indica, Momordica tuberose, Oxalis corniculata, Picrorhiza scrophulariiflora, Salvia officinalis, etc[12].

Bauhinia variegata

Bauhinia variegata is a species of flowering plant in the legume family, Fabaceae. It is native to an area from China through Southeast Asia to the Indian subcontinent. Common names include **orchid tree** (though not belonging to the family Orchidaceae) and **mountain ebony**[13].

Scientific Classification

Kingdom: Plantae

Order: Fabales

Family: Fabaceae

Genus: *Bauhinia*

Species: ***B. variegata***

It is a small to medium-sized tree growing to 10–12 metres (33–39 ft) tall, deciduous in the dry season. The leaves are 10–20 centimetres (3.9–7.9 in) obcordate shaped, long and broad, rounded, and bilobed at the base and apex. The flowers are conspicuous, bright pink or white, 8–12 centimetres (3.1–4.7 in) diameter, with five petals. Pollens are elongated, approximately 75 microns in length.

Geographical Source:

variegata is native to temperate and tropical China, the Indian Sub-continent (i.e. Bhutan, India, Nepal and Pakistan) and south-eastern Asia (i.e. Laos, Myanmar, Vietnam and Thailand).

Chemical constituents:

The chemical constituents isolated so far from the plant are β-sitosterol, kaempferol-3-glucoside, tannins, carbohydrates, amides, reducing sugars, vitamin C, crude protein, fibers, calcium,

phosphorus, quercetin, rutin, quercitrin, apigenin, apigenin-7-O-glucoside, heptatriacontan-12, 13-diol and dotetracontan-15-en-9

Uses:

Kachnar is a local name in the Indian subcontinent for the edible buds collected from the tree; it is widely used as an ingredient in many subcontinent recipes. Traditional kachnar curry is prepared using kachnar buds, yogurt, onions and native spices. Kachnar buds are also eaten as a stir-fried vegetable and used to make achaar, a pickle in many parts of the Indian sub-continent.[6] It shows a good antioxidant and anticancer activity.

In Nepal, the plant is known as *koirala* and the flower is known as *koirala ko phool* in Nepali language. The flower and the buds are used to make Nepalese style achaar. The flowers and buds are boiled for a while and then mixed with roughly mashed boiled potato, chopped green chilies, chopped onions, fresh peas, coriander leaves and spices like *timmur*, salt, pepper, turmeric powder, roasted sesame powder (*til ko chhop*), cumin powder and coriander powder. Heated mustard oil and fenugreek seeds is then added to the mixture and then lemon juice or lime juice concentrate (*chuk amilo* in Nepali) is then added as per taste. The achar is then served instantly. The achar is important part of the meal served during the Ghode Jatra festival**(14)**.

Macrotyloma Uniflorum

(Horse gram, kulthi bean, hurali, Madras gram)

Macrotyloma Uniflorum is one of the lesser known beans. The horse gram is normally used to feed horses, though it is also commonly used in cooking. In traditional Ayurvedic cuisine, horse gram is considered a food with medicinal qualities. It is prescribed for persons suffering from jaundice or water retention and as part of a weight loss diet. Although rich in proteins (20%), due to less acceptable taste and flavor of cooked products, it is consumed only by the farming community and low-income groups. Thus, it has remained an underutilized food legume. Horse Gram is mainly cultivated in India. It is also cultivated in Sri Lanka, Malaysia, West Indies etc. In India, this is grown in the states of Karnataka, Andhra Pradesh, Orissa, Tamil Nadu, Madhya Pradesh, Chhattisgarh, Bihar, West Bengal, Jharkhand and in the foot hills of Uttaranchal and

Himachal Pradesh. It is consumed as a whole seed, as sprouts, or as whole meal in India, popular in many parts of India. Medical uses of these legumes have been discussed[15].

Morphological Description

Horse gram is a short day, twining, succulent, annual climbing herb which has trifoliate seeds, white coloured flowers, long linear pubescent pods with curved beak, flattened small seeds with light red, brown, grey, black or mottled testa with photo and thermo-sensitive nature. Twining annual or perennial forming dense growth 30-60 cm high. Trifoliate seeds 3-7 cm long and 2-4 cm wide. Yellow flowers with violet blot on the standard. Pods 6-8 cm long and 4-8 mm wide with 6-7 seeds. Var. uniflorum is cultivated annual form which has wider pods. It is drought resistant but cannot withstand waterlogging. The mature herbage contains 18 per cent CP in DM.

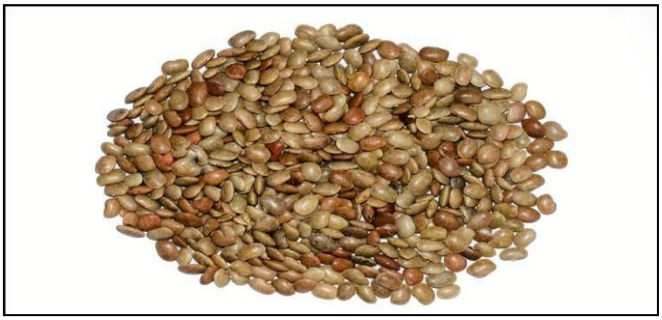
Systemic Classification

Kindom: Plantae
Family: Fabaceae
Genus: *Macrotyloma*
Species: *M.Uniflorum*
Synonyms:Horse gram

Vernacular Names:-

English: Horse Gram

Hindi: Kulthi Ki Dal.
Tamil : Madras gram



Geographical distribution, Origin, and habitat

It is now cultivated in Asia, Africa, West Indies and southern USA as a pulse crop and for fodder Horse gram is an extremely drought-resistant crop. Moderately warm, dry climatic conditions are suitable for its optimum growth. It does not grow well on higher altitudes because of cool and wet climate. Horse gram can be cultivated up to an altitude of 1000 m above the sea level. The temperature range of 25 °C-30 °C and relative humidity between 50% and 80% is optimum for

its growth. Heavy rains during the initial stages of crop growth affect nodule formation owing to poor aeration in the soil. A well-distributed rainfall of about 800 mm is sufficient for its successful cultivation, but it performs well even under low rainfall areas.

It is native to the old world tropics and indigenous to India. Archaeological investigations have revealed the use of horse gram as food especially in India as origin around 2000 BC. Horse gram belongs to the genus Macrotyloma and contains 25 species indigenous to Africa and Asia. Presence of wild or naturalized horse gram is recorded in Africa (Central, East and Southern Africa) and India. The primary center of origin and use of horse gram as a cultivated plant is in the plains and hills of low altitude extending southwards in the Western Ghats in South West India. During the Neolithic period, through counter-migration of human beings, its cultivation will be diffused to the northern and western parts of the Indian subcontinent. It is generally grown in sub-humid to semi-arid climates with annual rainfall 300–600 mm, and also with less than 30 cm rainfall as a dry-land crop up to an elevation of 1800 m from mean sea level. The optimum temperature range for its growth is 25 °C to 32 °C; it can tolerate temperatures up to 40 °C, but the growth rate declines markedly below 20 °C

Phytochemical Constituents:

Horse gram has been recognized as potential source of protein and other nutrients. It has high nutritional value equivalent to other commonly grown pulse crops in all aspects and also an excellent source of iron, molybdenum and calcium. Horse gram seed contains carbohydrate (57.2% w/w), protein (22% w/w), dietary fiber (5.3% w/w), fat (0.50% w/w), calcium (287 mg), phosphorus (311 mg), iron (6.77 mg) and calories (321 kcal) as well as vitamins like thiamine (0.4 mg), riboflavin (0.2 mg) and niacin (1.5 mg) per 100 grams of dry matter. However, several factors like the genotype, soil, fertilizer application, cultural practices, weather and climatic factors, post-harvest handling and storage can directly or indirectly affect the nutritional quality. Horse gram seed is low in fat and is excellent sources of protein, dietary fiber, a variety of micro-nutrients and phytochemicals still it has remained an underutilized food legume, consumed only by the farming communities of inaccessible areas and low-income groups.

Medicinal Uses

Scientists from the Indian Institute of Chemical Technology have found that unprocessed raw horse gram seeds not only possess antihyperglycemic properties, but also have qualities which reduce insulin resistance.

It is used in renal ailments as diuretic and to treat blood pressure.

In the treatment of nephrotic syndrome. Hepatitis, gall bladder abnormalities, and urinary disorders.

For kidney and urinary tract disorders (stones, nephritis, urethritis, infections, renal insufficiency/injury, etc)

Ethnobotanical Importance

Macrotyloma Uniflorum have held an important place inherbal medicine in both Brazil and India for many years. One of Brazil's leading medical herbalists, reports ervatostão is "a plant medicine of great importance, extraordinarily beneficial in the treatment of liver and kidney disorders." The seeds juice is used in treating scanty urine, and internal inflammation disorders.
In traditional medicine, ***Macrotyloma Uniflorum*** species have been used to treat ailments such as infections, rheumatism and inflammation. ***Macrotyloma Uniflorum*** extracts also have immunosuppressive effects. ***Macrotyloma Uniflorum*** has been recorded in Trinidad and Tobago as being used as a traditional treatment for hypertension and for the treatment of kidney stones in India.
InIndo-Nepal Himalayan Terai region, the tribals harvest this plant for medicinal purposes, mainly for flushing out the renal system, and to treat seminal weakness and blood pressure
Medicine use to as a diuretic, for all types of liver disorders (including jaundice and hepatitis), gallbladder pain and stones, urinary tract disorders, renal disorders, kidney stones, in some part of Rajasthan.
It is employed in Brazilian herbal medicine to stimulate the emptying of the gallbladder, as a diuretic, for all types of liver disorders (including jaundice and hepatitis), gallbladder pain and stones, urinary tract disorders, renal disorders, kidney stones, cystitis, and nephritis[(16)].

LITERATURE REVIEW

Reported Pharmacological Activities of Bauhinia variegata

Anthelmintic activity

- Bairagi, *et al*. **(2012)** investigated "In vitro anthelmintic activity of Bauhinia variegata bark (Leguminosae)"[17].
- Mali RG, *et al*. **(2008)** investigated Evaluation of *Bauhinia variegata* Linn stem bark for anthelmintic and antimicrobial properties[18].

Hepatoprotective activity:

- Bodake, *et al*. **(2007)** investigated "Hepatoprotective properties of Bauhinia variegata bark extract"[19].

Haematinic activity

- Dhonde SM, *et al*. **(2007)** investigated Haematinic activity of ethanolic extract of stem bark of *Bauhinia variegata* Linn.[20]

Wound Healing Activity

- Neto LGN, *et al*. **(2007)** investigated Effect of the Lectin of *Bauhinia variegata* and Its Recombinant Isoform on Surgically Induced Skin Wounds in a Murine Model. [21]

Anti- inflammatory activity:

- Koteswara RY, *et al*. **(2008)** investigated "Anti- inflammatory activity of flavanoids and a triterpene caffeate isolated from Bauhinia variegate"[22].
- Rao YK, *et al*. **(2008)** investigated Antiinflammatory activities of flavonoids and a triterpene caffeate isolated from *Bauhinia variegata*.[23]
- Mohamed MA, *et al*. **(2007)** investigated Evaluation of antinociceptive and anti-inflammatory activities of a new triterpene saponin from *Bauhinia variegata* leaves.[24]
- Yadava RN and Reddy VM **(2003)** investigated Anti-inflammatory activity of a novel flavonol glycoside from the *Bauhinia variegata* Linn.[25]

Antioxidant activity:

- Rajani P.G. and Ashok P (2009) investigated "In vitro antioxidant and antihyperlipidemic activities of Bauhinia variegata Linn"[26].

Nephroprotective activity:

- Panda P.K., *et al*. **(2011)** investigated "Nephroprotective effect of Bauhinia variegata (Linn.) whole stem extract against cisplatin-induced nephropathy in rats"[27].

Sharma RK, *et al*. **(2011)** investigated Effect of ethanolic and aqueous extracts of *Bauhinia variegata* Linn. on gentamicin-induced nephrotoxicity in rats[28].

Immunomodulatory activity:

- Patil J.K., *et al*. **(2010)** investigated "In vitro immunomodulatory activity of extracts of *Bauhinia variegata* Linn. stem bark on human neutrophils"[29].

Antitumour activity:

- Kanak S and Verma Anita K **(2012)** investigated Evaluation of antimicrobial and anticancer activities of methanol extract of *in vivo* and *in vitro* grown *Bauhinia variegata* L[30].
- Rajkapoor B., *et al*. **(2011)** investigated "Antitumour activity of Bauhinia variegata on Dalton's ascetic lymphoma"[31].
- Sonam P, *et al*. **(2009)** investigated Effects of *Bauhinia variegata* bark extract on DMBA induced mouse skin carcinogenesis.[32]
- RajKapoor B, *et al*. **(2006)** investigated Chemoprevention and cytotoxic effect of *Bauhinia variegate* against N-nitrosodiethylamine induced liver tumors and human cancer cell lines[33].

Antihyperlipidemic activity:

- Kumar D, *et al*. **(2011)** investigated Effect and evaluation of antihyperlipidemic activity of fractions of total methanol extract of *Bauhinia variegata (Linn.)* leaves on Triton WR-1339 (Tyloxapol) induced hyperlipidemic rats[34].

Antimicrobial activity:

- Dhale AD *et al*. **(2011)** investigated Phytochemical screening and antimicrobial activity of *Bauhinia variegata* Linn[35].
- Parekh J, *et al*. **(2006)** investigated Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* Linn. Bark[36].
- Sharma RN and Saxena VK **(1996)** investigated In vitro antimicrobial efficacy of leaves extracts of *Bauhinia variegata* Linn[37].

Antiobesity activity:

- Balamurugan G and Muralidharan P **(2010)** investigated Antiobesity effect of *Bauhinia variegata* bark extract on female rats fed on hypercaloric diet[38].

Antiulcer activity:

- Rajkapoor B, *et al*. **(2003)** investigated Antiulcer effect of *Bauhinia variegata* Linn. in rats[39].

Antidiabetic activity:

- Frankish N, *et al*. **(2010)** investigated Enhancement of Insulin Release from the ß-Cell Line INS-1 by an Ethanolic Extract of *Bauhinia variegata* and Its Major Constituent Roseoside[40].

- Koti BC, *et al*. **(2009)** investigated Effect of *Bauhinia variegata* bark extract on blood glucose level in normal and alloxanised diabetic rats[41].

Toxicity Study:

- Rasika Kolhe *et al*. **(2014)** investigated Chronic toxicity study of stem bark powder of Kanchanara (Bauhinia variegata L.) in albino rats[42].
- Singh KL, *et al*. **(2012)** investigated Toxicity of *Bauhinia variegata* and *Mimusops elengi* with plant molluscicides against *Lymnaea acuminate*[43].

Reported Pharmacological Investigations of *Macrotyloma Uniflorum*

Hepatoprotective Activity

- Parmar H.B, ***et al*** **(2012)** investigated hepatoprotective activity of *macrotyloma uniflorum* Seeds extract on paracetamol and d-galactosamine induced liver toxicity in albino rats[44].

AntioxidantnActivity:

- Ravishankar VP ***et al*** **(2012)** investigated in vitro antioxidant activity of ethanolic seed extracts of *macrotyloma uniflorum*[45].
- Renu singh, ***et al*** **(2012)** investigated In-vitro antioxidant and free radical scavenging activity of *Macrotyloma uniflorum*[46].
- Muthu AK, ***et al*** **(2006)** investigated Antioxidant potential of methanolic extract of *Macrotyloma Uniflorum*[47].
- Kottai Muthu A K, ***et al*** **(2006)** investigated Antioxidant potential of methanolic extract of *Dolichos biflorus* Linn[48].

Antidiabetic & Hypoglycemic Activity:

- Raj DM and Reddy KB. (2016) investigated Synergistic effect of *Camellia sinensis* and *Macrotyloma uniflorum* as anti- hyperlipidaemic and antidiabetic activity[49].
- Parthsarthi PB and Saxena Y. (2013) investigated Effect of *Dolichos biflorus* on blood sugar and lipids in diabetic rats[50].
- Tiwari, AK, Manasa K, Kumar DA and Zehra A. (2013) investigated Raw horsegram seeds possess more *in-vitro* antihyperglycaemicactivities[51].
- Lakxmi H. Gupta, **et al** (2011) showed Antidiabetic potential of α-amylase inhibitor from the seeds of *Macrotyloma uniflorum* in streptozotocin-nicotinamide-induced diabetic mice[52].

Anti-obesity activity:

- Sengupta K, Mishra AT, Rao MK, Sarma KV, Krishnaraju AV and Trimurtulu G.(2012) investigated Efficacy of an herbal formulation LI10903F containing *Dolichos biflorus* and *Piper betle*extracts on weight management[53].
- Kumar DS, Prashanthi G, Avasarala H, Banji D. (2013) investigated Antihypercholesterolemic effect of *Macrotyloma uniflorum* (Lam.) extract on high-fat diet-induced hypercholesterolemia[54].

- Bharathi V and Anand V. (2014) investigated Antiobesity effect of *Macrotyloma uniflorum* leaves extract on rats fed on a hypercaloric diet[55].
- Bhuvaneshwari S. (2014) investigated Anorectic activity of *Dolichos biflorus*[56].

Anti-urolithiatic activity :

- Sharma N, Bisht SS, Gupta S, Panda AK and Rana M. (2018) investigated Analysis of proteomic diversity and calcium binding protein (s) in seeds of horse gram *(Macrotyloma uniflorum*)[57].
- Ahmed S, Hasan MM and Mahmood ZA. (2016) investigated Inhibition of calcium oxalate crystals growth by *Macrotyloma uniflorum*[58].
- Chaitanya DA, Kumar MS, Reddy AM, Mukherjee NS, Sumanth MH and Ramesh A. (2010) investigated Anti urolithiatic activity of *Macrotyloma uniflorum* seed extract on ethylene glycol induced urolithiasis in albino rats[59].
- Peshin A and Singla SK. (1994) investigated Anticalcifying properties of *Dolichos biflorus* (horse gram) seeds[60].

Diuretic Activity:

- Ashraf J, Baig SG, Ahmed S and Hasan MM. (2018) investigated Analgesic, anti-inflammatory and diuretic activities of *Macrotyloma uniflorum* (Lam.)[61].
- Ravishankar K and Priya PS, (2012) investigated Evaluation of diuretic effect of ethanolic seed extracts of *Macrotyloma uniflorum* and *C. melo* in rats [62].

Analgesic, Antinociceptive and Anti-inflammatory Activity:

- Fatima SA, Baig SG, Hasan MM and Ahmed S. (2018) investigated Analgesic and anti-inflammatory activities of fixed oil of *Macrotyloma uniflorum* (Lam.) Verdc. in mice and rats[63].
- Rajagopal V, Pushpan CK and Antony H. (2017) investigated Comparative effect of horse gram and black gram on inflammatory mediators and antioxidant status[64].
- Giresha AS, Narayanappa M, Joshi V, Vishwanath BS and Dharmappa KK. (2015) investigated Human secretory phospholipase A2 (spla2) inhibition by aqueous extract of *Macrotyloma uniflorum* (seed) as anti-inflammatory activity[65].
- Mathew E, Sindhu G and Helen A. (2014) investigated *Dolichos biflorus* exhibits anti-inflammatory and antioxidant properties in anacute inflammatory model[66].

Anthelmintic activity :

- Sree VK, Soundarya M, Ravikumar M, Reddy TR and Devi NK. (2014) investigated *In-vitro* screening of *Macrotyloma uniflorum* extracts for antioxidant and anthelmintic activities[67].
- Philip A, Athul PV, Charan A and Afeefa TP. (2009) investigated Anthelmintic activity of seeds of *Macrotyloma uniflorum*[68].

Antitumor and Cytotoxic Activity:

- Chakraborty G, Manna K, Debnath B, Somraj SW and Goswami S. (2018) investigated Phytochemical analysis, anti-oxidant and cytotoxic activity of seed coat of *Macrotyloma uniflorum* in different solvents[69].
- Chen YW, Huang SC, Lin-Shiau SY and Lin JK. (2005) investigated Bowman-Birk inhibitor abates proteasome function and suppresses the proliferation of MCF7 breast cancer cells through accumulation of MAP kinase phosphatase-1[70].

AIM AND OBJECTIVE

AIM

The aim of the present work is to develop a liposome of ***Bauhinia Variegata*** **and** ***Macrotyloma Uniflorum*** **(Lam) herbal plants extract** and evaluate the formulation as per WHO guidelines.

OBJECTIVES

1. Raw material analysis

To collect and authentication of the Plant materials

Phytochemical Investigations:

Extraction of plant material

To carryout phytochemical tests of extracts.

To isolate phytoconstituents from the extracts

2. Preformulation study

Preformulation studies of liposomes

3. Standardization of the liposomes

To standardise the physico-chemical parameters of liposomes.

To analyse the presence of phytoconstituent in the liposomes.

To validate UV and HPLC method for the quantitative estimation of liposome formulation during solubility study, in vitro study, in vivo study and stability study.

4. Stability studies

To Establish stability of the liposome formulation as per ICH guidelines.

To establish the Nephroprotective activity of liposome formulation.

PLAN OF WORK

1. Collection and Authentication

2. Processing of raw materials:

Drying and Size reduction of plant materials

3. PHYTOCHEMICAL INVESTIGATIONS:

Preparation of Extract
Preliminary phytochemical screening of extracts
Isolation of phytoconstituents from the extracts by
Thin Layer Chromatography
HPLC - analysis

4. PRE FORMULATION STUDY

Solubility of isolated compound by "Shake flask method"

Partition coefficient of isolated compound by Separating flask in suitable solvent

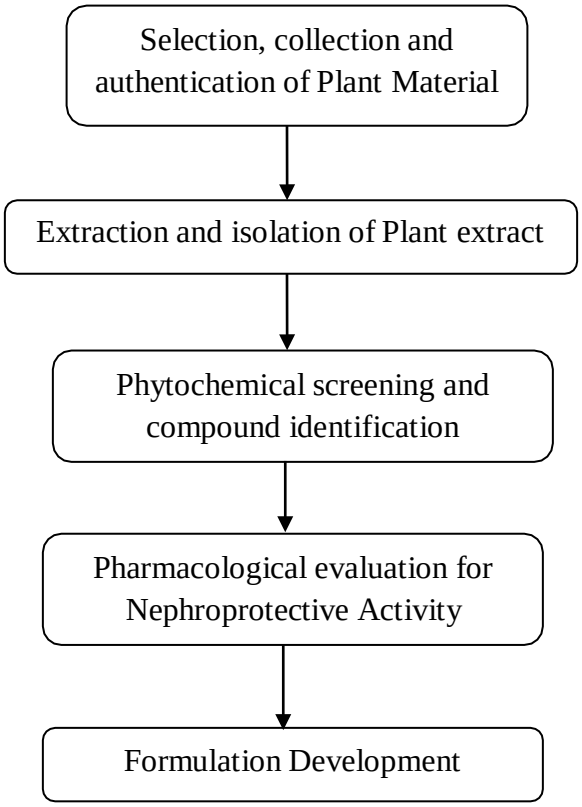
Isolated compound -excipients compatible study by FT-IR spectroscopy and DSC

5. DEVELOPMENT OF LIPOSOME FORMULATION

Formulation of liposome by thin layer film hydration method

6. EVALUATION OF LIPOSOME FORMULATION

DEVELOPMENT OF FORMULATION


Selection, collection and authentication of Plant Material
Extraction and isolation of Plant extract
Phytochemical screening and compound identification
Pharmacological evaluation for Nephroprotective Activity
Formulation Development

MATERIALS AND METHODS

PHYTOCHEMICHAL INVESTIGATIONS [37]

Collection and Authentication of Plant

Plant materials will be collected and then authenticated as *Bauhinia variegata and Macrotyloma Uniflorum* by Recognized botanical department, Rajasthan.

Drying and Size reduction

1. Drying: Plant materials will be dried.

2. Size reduction: The plant seeds will be powdered in iron mortar & pestle. The coarse powder will be mesh size to get moderately coarse powder.

Extraction of the processed plant material

Bauhinia variegata leaves powder will be extracted by aqueous extraction method in soxhlet apparatus at 35-40^{0}C temperature, 500g of the powder will be mixed with distilled water. The mixture will be filtered in Buchner funnel and dried over water bath till dryness. Then percentage yield will be determined[42].

Macrotyloma Uniflorum seeds powder will be extracted by aqueous extraction method in soxhlet apparatus at 35-40^{0}C temperature, 500g of the powder will be mixed with distilled water. The mixture will be filtered in Buchner funnel and dried over water bath till dryness and percentage yield will be determined.

Preliminary Phytochemical screening (37)

Preliminary tests will be carried out for the presence or absence of phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Glycosides, Reducing sugars, Saponins, sterols, Anthocyanins, Terpenes and Tannins. A description of methods adopted for performing the tests are summarized below.

Tests for Alkaloids

A. Mayer's Test:

The Extract to be tested will be treated with a few drops of dilute 2N HCL and 0.5 ml Mayer's reagent .White precipitate will be obtained which confirmed the presence of alkaloids.

B. Wagner's Test

The extract will be treated with few drops of 2N HCL and 0.5 ml Wagner's reagent. Brown flocculent precipitate will be obtained which confirmed the presence of alkaloids.

C. Dragendorff's Test

Extract will be treated with few drops of dil. 2N HCL and 0.5 ml Dragendroff's reagent. Brown precipitate will be obtained which confirmed the presence of alkaloids.

A. Hager's Test

The extract will be treated with few drops of dilute 2N HCL and 0.5 ml Hager's reagent. Yellow colored precipitate will be obtained which confirms the presence of alkaloids.

Test for Anthocyanins

a) To 5ml of extract 2M sodium hydroxide will be added drop wise. Absence of blue green color indicated the absence of anthocyanins.

b) To 5ml of extract dilute sodium acetate solution and small quantity of ferric chloride will be added. Absence of reddish brown color indicated the absence of anthocyanins.

Test for Carbohydrates

A. Molisch's test

It will be performed for the presence of carbohydrates. About 1 ml of 10%alcoholic solution of α-napthol will be added to the extract and mixed. Then 1ml of concentrated sulphuric acid will be carefully poured along the sides of the test tube violet ring formed at the junction of two liquid which is considered positive test for carbohydrates.

Test for Flavonoids

A. Shinoda test

About 5-10 drops of dilute HCl will be added to 0.5 ml of extract. Small piece of magnesium will be added to it. Appearance of pink, reddish pink or brown coloration will be considered as positive test.

B. A small portion of extract will be treated with neutral ferric chloride solution. Appearance of green to blue color will be taken as positive test for flavonoids.

C. Small portion of extract will be treated with few drops of 10 % lead acetate solution. Appearance of yellow precipitate will be considered as positive test for flavonoids.

D. About 10 ml of solution of the extract will be hydrolyzed with dilute sulphuric acid. This will be extracted with ether and divided into two portions. To one portion 1ml dilute ammonia solution will be added, a greenish color confirms the presence of flavonoid. To other portion 1ml dilute Sodium carbonate solution will be added. A pale yellow color confirms the presence of flavonoids.

Test for Glycosides

A. The dried extract will be dissolved in glacial acetic acid and few drops of ferric chloride will be added followed by concentrated sulphuric acid. A bluish green precipitate will be considered to be a positive test for glycosides.

Test for reducing Sugar

A. Fehling's test

About 5ml of solution of extract will be heated with equal volumes of Fehling's solution A & B. Transition of colour from blue through green to reddish orange confirms the presence of reducing sugars.

B. Benedict's test

About 5 ml of solution of the extract will be heated with 5 ml of Benedict's reagent. A green, yellow or orange red precipitate will be considered as a positive test for reducing sugars.

Test for saponins

A. Foam's test

A small amount of dry extract will be boiled with water and allowed to cool. It will be then shaken vigorously for a minute. The formation of persistent honey comb like froth will be considered as a positive test for saponins.

Test for Sterols and Terpenoids

A. Liebermann–Burchard test

A small portion of extract will be dissolved in chloroform and 2ml of Liebermann- burchard reagent will be added. Appearance of bluish green will be considered as positive test for sterols and pink or violet coloration will be considered as positive test for Terpenoids.

B. Salkowski test

A small portion of extract will be dissolved in chloroform and treated with an equal volume of concentrated sulphuric acid. A Red to purple color formation will be considered as a positive test for Terpenoids.

Test for Tannins

A. A small portion of extract will be treated with 5%ferric chloride solution. Appearance of green to blue color will be taken as a positive test for tannins.

B. Small portion of extract will be treated with lead acetate. Appearance of creamy precipitate will be considered as a positive test for tannins.

Test for Proteins

A. Biuret test: A small portion of extract will be treated with Biuret reagent. Appearance of violet color indicates positive test for proteins[71, 72].

PREFORMULATION STUDIES[73]

Preformulation study will be done prior to developing a Liposome formulation as it helps in understanding various physicochemical characteristics of drugs and excipients.

Particle size: - The particle size of liposome will be determined by zeta sizer instrument. This instrument containing Malvern PCS software. Before taking the result of sample solution of the sample will be diluted with distilled water. The distilled water not interferes with result. Then after dilution the result were taken. The particle size must be required in nano range some time it goes to micron range if multilamellar vesicle are present. The particle size of sample solution will be determined by using light scattering technique and by transmission electron microscope. If the particle size of liposome increases then decrease the uptake and bioavailability of drug. The particle size is most important, the particle size of liposome in nano range are having more effective drug delivery as compare to micron range.

Zeta Potential: - The zeta potential means the charges which are present on the surface of liposome. The many time the charge is present on the surface of liposome. This charge is come due to the component or ingredient which will be used during the manufacturing. The zeta potential of liposome will be taken in zeta sizer instrument having Malvern software. The analysis of sample was carried out at 25^0 C with the angle of detection 90^o. The ideal zeta potential value must be required in range between +30 to -30mV. These ranges prevent the aggregation of liposomal particle.

Solubility of Liposome formulation in various solvents

An excess amount of Liposome formulation will be added in microcentrifuge tube (MCT) containing 1 ml solvent and vortexed it for 72 h at room temperature (25 ± 1 °C) with the help of Remi CM-101 cyclomixer. After 72 h, an excess amount of drug will be removed by centrifuging (Remi R8C Laboratory Centrifuge) the mixtures at 3000 rpm for 10 min. 10 µl supernatant will be taken in another MCT and volume made up to 1 ml with methanol. This mixture will be vortexed prior to filtration using 0.22-µm nylon filter. The absorbance will be taken at 230 nm after suitable dilution and finally, the unknown amount of drug dissolved in that particular solvent will be determined using previously developed calibration plot (Parveen et al. 2011).

Partition coefficient of Liposome formulation

Partition coefficient will be performed in a 2 ml micro centrifuge tube. Each ml of n-octanol and double distilled water will be taken in this tube. After adding 10 mg drug into this mixture, the tube will be fixed horizontally on a Remi cyclomixer with the help of adhesive tape. The mixture will be vortexed at room temperature for 3 hours and then stored overnight in vertical position. Thereafter 10 µl of octanol (octanol is lighter than water) will be taken out with the help of micropipette and suitably diluted with methanol to take absorbance at 230 nm.

Entrapment Efficiency: For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation will be determined (w) by UV spectrophotometer at 254 nm. A standard calibration curve of drug will be plotted for this purpose. The amount of drug in supernatant will be then subtracted from the total amount of drug added during the preparation (W). Effectively, (W-w) will give the amount of drug entrapped in the liposome.

% Drug Entrapment = (W-w/W) × 100

Preparation of calibration curve by UV spectrophotometric method in methanol for the solubility study and partition coefficient determination

10 mg Liposome formulation will be dissolved in 100 ml methanol in a volumetric flask to obtain 100 µg/ml concentration of primary stock solution. From this stock solution aliquots will be taken out with the help of a micropipette and suitably diluted to get the serial dilutions; 0.1, 0.5, 1, 2, 3 and 4 µg/ml. Absorption of these serial dilutions will be taken at 231 nm. All the dilutions will be prepared thrice and readings will be taken in triplicate (n=3).

Preparation of calibration plot by high performance liquid chromatography (HPLC) for in vitro release study, ex vivo permeation study, pharmacokinetic study and stability study

To develop a calibration plot of Liposome formulation, a primary stock solution of 50 µg/ml will be prepared by dissolving the drug in HPLC grade methanol.

Serial dilutions of concentrations like 40, 100, 200, 300, 500, 1000 and 10000 ng/ml will be prepared from the primary stock solution. The aliquots will be taken from the primary stock and the desired volume will be made up using HPLC grade methanol. The coefficient of correlation values (r^2) of developed calibration plot for non-biological samples will be found to be 0.997 and the linear equation generated from this calibration plot will be used to quantify the unknown concentration of Liposome formulation.

Method of validation

The developed HPLC method will be validated according to ICH guidelines and linearity, sensitivity, accuracy, precision, system suitability and robustness will be determined.

LIPOSOME DEVELOPMENT[74-75]

Preparation of liposome by thin layer film hydration method

This method involves making a thin lipid film in a round-bottom flask by the removal of organic solvent. Upon the addition and agitation of the dispersion medium, heterogeneous liposomes are formed. Finally, after extrusion through polycarbonate membranes, homogeneous small liposomes are obtained.

Method: By using Thin film hydration method the multi lamellar liposome will be prepared. Herbal drug, soya lecithin and cholesterol will be dissolved in chloroform and methanol mixture in ratio (9:1). These above solutions pour in round bottom flask of rotary flash evaporator. In rotary flash evaporator the organic solvent evaporates at 600c, for 15min. at 90rpm. After evaporating organic solvent thin layer which will form on inner surface of round bottom flask. These thin layers dried overnight by using vacuumed oven. Then this thin lipid layer suspension in phosphate buffer saline (PBS) having PH-7.4 by vertexing for 10min.and then it will be allowed to hydrate for 1hr at 70oc, 90 rpm. Then this liposomal suspension centrifuge by using ultra-centrifugation Machin at 3000rpm for 30 min. Then the settle liposome again centrifuge in PBS. Then this suspension of liposome sonicates for 15 min at 65oc to get small unilamellar vesicle (SUV).[74-75].

Pharmacokinetic analysis

The plasma concentrations of Liposome formulation at different time interval will be calculated from the HPLC data and various pharmacokinetic parameters will be determined by a pharmacokinetic software (PK Functions for Microsoft Excel, Pharsight Corporation, Mountain View, CA). The area under the curve, determined by software will be used to calculate direct transport percentage (DTP) and drug targeting efficiency % (DTE%) of Liposome formulation.

DATA ANALYSIS

Experiments will be carried out at least in triplicates and data are expressed as the mean ± standard deviation (S.D). In the pharmacodyanamic study, one way ANOVA will be applied to test the differences between treatments which will be followed by Tukey's multiple comparisons test. Differences will be considered significant at $p < 0.05$.

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